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(54) Title: INFANT OR FOLLOW-ON FORMULA

(57) Abstract: The formula of the invention, intended both for infants and young children, comprises modified sweet whey proteins free or almost free of CGMP, and at least one probiotic. The invention also pertains to methods for promoting physical development, improving gastro intestinal comfort and developing a healthy gut microflora in infants or young children by fully or partly feeding them with the said formula.

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## INFANT OR FOLLOW-ON FORMULA

**Field of the invention**

5      The present invention relates to a nutritional composition intended for infants and/or young children, as well as to methods for improving gastrointestinal comfort, developing a healthy gut microflora, and promoting the physical development of infants and/or young children by partly or fully feeding said infants or children with the aforementioned nutritional composition.

10     **Background of the invention**

The composition of human milk serves as a valuable reference for improving infant formula. However, human milk contains living cells, hormones, active enzymes, 15 immunoglobulins and components with unique molecular structures that cannot be replicated in infant formula. Unlike human milk, infant formula must remain stable on the shelf for up to thirty-six (36) months. These fundamental differences between human milk and infant formula often mandate differences in the composition to achieve similar clinical outcome.

20     The study of human milk components has stimulated many investigations into what constituents may be added to an improved infant formula. Greater knowledge of the composition of human milk affords the opportunity to design infant formulas that are closer in composition to human milk. However, it becomes increasingly apparent that 25 infant formula can never exactly duplicate human milk. Many constituents in human milk are bioactive and because of synergies among these components, there is little reason to believe that the same compound would have the same bioactivity in infant formula. The likelihood of this possibility is further diminished when the impact of heat treatment for sterilization and long-term storage of the formula is considered.

30     The composition of human milk differs appreciably from that of other species and much attention has been paid to the various components. Several investigators have reported 35 on the nucleotide content of milk from humans. Numerous publications have also discussed various lipid, oil or fat blends for use in an artificial nutritional for human infants.

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There is a need for new formulae, providing to the infant or the young child a nutritional contribution with a unique combination of protective nutrients, especially ensuring growth and metabolic patterns similar to those of breastfed infants, thus resulting in similar health characteristics in later childhood and adulthood.

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### **Summary of the Invention**

The present invention therefore pertains to formulae intended both for infants and young children. The formula of the invention comprises comprising a source of proteins, a source of lipids, a source of carbohydrates and a probiotic, wherein at least 40% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.

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The present invention also provides a method of promoting physical development of an infant or a young child consisting in fully or partly feeding the infant or child with the said formula.

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The present invention further provides a method of improving gastro-intestinal comfort of an infant or a young child consisting in fully or partly feeding the infant or child with the said formula.

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The present invention further provides to a method of developing a healthy gut microflora in an infant or a young child consisting in fully or partly feeding the infant or child with the said formula.

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### **Detailed Description of the Invention**

In the present specification, the following words are given a definition that must be taken into account when reading and interpreting the description, examples and claims.

30

Infant: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(a), the term "infants" means children under the age of 12 months. This definition is adopted in the present specification.

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Young Children: according to the Commission Directive 91/321/EEC of 14 May 1991 on infant formulae and follow-on formulae, article 1.2(b), the term "young children"

means children aged between one and three years. This definition is adopted in the present specification.

Infant formulae: according to the Commission Directive 91/321/EEC of 14 May 1991

5 on infant formulae and follow-on formulae, article 1.2(c), the term "infant formula" means foodstuffs intended for particular nutritional use by infants during the first four to six months of life and satisfying by themselves the nutritional requirements of this category of persons. This definition is adopted in the present specification. It has to be understood that infants can be fed solely with infant formulas, or that the infant formula

10 can be used by the carer as a complement of human milk. It is synonymous to the widely used expression "starter formula".

Follow-on formulae: according to the Commission Directive 91/321/EEC of 14 May

15 1991 on infant formulae and follow-on formulae, article 1.2(d), the term "follow-on formulae" means foodstuffs intended for particular nutritional use by infants aged over four months and constituting the principal liquid element in a progressively diversified diet of this category of persons. This definition is adopted in the present specification. This definition is adopted in the present specification.

20 Probiotic: according to the paper Probiotics in Man and Animals, J. Appl Bacteriol. 66: 365-378, a probiotic is defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

25 According to a first aspect of the invention, there is provided a nutritional formula for infants (including a starter composition) or young children. As already mentioned, it is an object of the invention to provide a unique combination of protective nutrients ensuring growth and metabolic patterns similar to those of breastfed infants, with the intention of enabling similar health characteristics to be enjoyed in later childhood and adulthood.

30 Formulae of the invention present a reduced load on immature organs; and favour the natural growth of Bifidobacteria and other beneficial microflora in the large intestine as in breastfed infants.

35 Dietary protein provides the essential amino acids necessary for protein synthesis and growth and protein quality is as important as protein quantity. Until recently, it was

thought that in order to supply enough of the essential amino acids, formulae based on cows' milk needed a protein content significantly higher than that of the reference human milk. The protein content of regular whey-adapted formulae ranges from 2.1 to 2.6 g per 100 kcal, whereas the content of human milk ranges from 1.4 to 1.8 g per 100 kcal. Excess protein intake may induce metabolic stress on infant organs that have not fully developed. Following paediatric recommendations for lowering protein density of infant formulae, clinical trials in infants fed formulae containing protein densities between 1.6 and 2.0 g / 100 kcal have been reported. However, these attempts to lower protein content in a formula using traditional cow's milk protein sources or mixing the currently available fractions – casein and whey -, although demonstrating the principle was conceivable, failed to reproduce all the indices of human milk protein metabolism or to ensure the satisfactory growth of infants. For instance, results have shown a global plasma amino acid pattern different to that of breast-fed infants, depressed plasma tryptophan levels, elevated plasma threonine levels, delay in growth, and higher energy intake suggesting an increased fat deposition which may be responsible for obesity in later life.

However, if the amino acid pattern of a cow's milk-based infant formula can be made closer to that of human milk, the protein content of such a formula can be lowered to resemble that of the reference. A protein mixture of unique amino acid composition allowing the adaptation of the quantity of protein to a level closer to the average content of human milk has been developed according to an aspect of the present invention.

The protein fraction in cows' milk is a mixture of several proteins, which all have a different amino acid profile. Caseino-glyco-maclopeptide (CGMP) is a protein that is found in this fraction. It comes from the kappa-casein that is split up by proteolytic cleavage into 2/3 para-kappa-casein, an insoluble fraction that remains in the casein fraction and 1/3 CGMP, a soluble fraction that is found in the whey fraction. An original fractionation process of whey proteins has been developed and is explained in EP 880902; this process allows the removal of practically all the CGMP (a fraction rich in threonine and poor in tryptophan) from bovine whey thereby increasing the alpha-lactalbumin proportion (a fraction very rich in tryptophan). By combining this modified sweet whey (MSW) fraction with skim milk, and with the addition of some free L-histidine and L-arginine (in order to reach the minimum amounts of these amino acids required by EC Directive), the protein source of the formula according to the invention has an amino acid profile much closer to that of human milk, characterised in particular

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by comparable tryptophan and threonine levels, allowing the adaptation of its protein content to that of human milk.

The nutritional value of this protein mixture has been measured in rats. The results show (see table 1) that this formulation has a Protein Efficiency Ratio (PER), a nitrogen digestibility, a Biological Value (BV), and a Net Protein Utilisation (NPU) comparable to standard whey-adapted formulae.

Table 1

10

Nutritional parameters	Casein	standard whey-adapted formula	New formulation
PER	1.36	2.49	2.70
Relative PER (casein = 100%)	100.0	182.8	198.3
Digestibility (%)	96.7	92.8	91.4
BV	0.88	0.96	0.96
NPU (%)	85.4	88.8	87.5

Moreover, rats fed on a formulation containing MSW showed significant lower plasma threonine levels and increased plasma tryptophan levels, compared to rats fed on standard whey-adapted formulae.

15

The protein content of formulae according to the present invention is preferably no more than 2g/100 kcal, more preferably less than 1.85, most preferably between 1.8 and 1.85 g/100 kcal. This level is in line with recent data assessing protein requirements during early life, which has shown that recommendations for optimal protein intakes are lower than they have been reported in the past.

20

To ensure optimal protein synthesis, and therefore optimal growth, essential and semi-essential (i.e. essential only during infancy) amino acids need to be supplied in the same quantities as in human milk. Formulae according to the invention are preferably either whey enriched (casein / whey ratio set around 40/60) or, more preferably, whey predominant (casein / whey ratio preferably set at 30/70 or even more, such as 20/80). A preferred amino acid profile for formulae according to the invention is comparable to that of human milk (see table 2).

30

Table 2

Amino acid (g)	Human milk	Invention
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<i>/ 16 g N)</i>	<b>mean</b>	<b>lowest value</b>	<b>highest value</b>	<b>(representative values)</b>
Isoleucine *	6.4	5.7	6.8	5.8
Leucine *	11.5	11.0	11.9	11.9
Lysine *	7.9	7.4	8.4	10.0
Methionine *	1.7	1.3	2.1	2.5
Cystine **	2.3	1.7	2.9	2.4
Phenylalanine*	4.6	4.2	5.1	4.6
Tyrosine **	4.7	3.3	6.3	4.0
Threonine *	5.6	5.3	6.6	5.4
Tryptophan *	2.3	1.8	2.6	2.1
Valine *	6.8	5.9	8.0	5.9
Arginine **	4.2	3.5	4.9	4.5
Histidine **	2.8	2.4	3.8	2.5
Alanine	4.8	4.5	5.3	5.1
Aspartic acid	10.4	10.1	10.8	11.1
Glutamic acid	19.6	17.6	22.7	19.7
Glycine	3.2	2.8	3.6	2.7
Proline	10.2	8.9	11.2	7.8
Serine	5.6	5.0	5.9	5.3

All values corrected to 40% NH<sub>3</sub>

\* essential amino acids \*\* semi-essential amino acids

5 The proteins may be either intact or partially hydrolysed by a process such as that described in European Patent No 322589.

10 Preferably, the sole source of carbohydrates of the composition according to the present invention is lactose. Carbohydrates constitute an important source of energy in the diet of the newborn infant. Lactose is the natural carbohydrate in human milk. Most infants in good health can digest lactose adequately. Further, lactose is associated with stool acidity and the development of a *Bifidobacteria* and *lactobacilli* preponderant microflora in the large intestine similar to that of breastfed babies. This is thought to be important in suppressing the growth of undesirable bacteria in the large intestine. Moreover, lactose has been shown to enhance absorption and retention of calcium and probably other minerals. In a recent study, it has been shown that calcium absorption is 10% greater from a lactose-containing formula compared with the same formula in which the lactose was replaced by glucose polymers.

15

20 As previously mentioned, formulae according to the invention comprise at least one probiotic, in order to offer all infants, whatever their mode of delivery or their hygienic environment, the advantages of a protective intestinal flora.

Preferred probiotics are those which as a whole are safe, are L (+) lactic acid producing cultures and have acceptable shelf-life for products such as infant and follow-on formulae which are required to remain stable and effective for up to 36 months.

5 Examples of preferred probiotics are:-

*Bifidobacterium lactis*, first sold by Christian Hansen company;

10 *Streptococcus thermophilus* provided under the name TH4 by Chr. Hansen, Denmark;

*Lactobacillus paracasei rhamnosus GG* (ATCC 53103) provided by Valio Oy, Finland;

*Bifidobacterium longum BB536* provided by Morinaga Milk Industry Co. Ltd, Japan.

15 The probiotics according to the present aspect of the invention are preferably present in an amount of  $10^6$  to  $10^9$  cfu/ grams of dry product, preferably  $10^6$  to  $10^8$  cfu/g, and even more preferably  $2 \times 10^7$  cfu/grams of dry product.

20 The composition according to the present invention comprises at least one probiotic strain but combinations of different strains may also be used, particularly in follow-on formulae. If such a combination is to be used, it will preferably include at least one Bifidobacteria and at least one Lactobacillus. A particularly preferred combination is *Bifidobacterium longum BB536* and *Lactobacillus paracasei rhamnosus GG*.

25 The formulae of the present invention also comprise a source of lipids. Fat provides about half of the dietary energy and constitutes the major energy stores in the bodies of infants and young children. Presently, there is growing interest in the quality of the dietary lipid supply during infancy as a major determinant of growth, visual and neural development, and long-term health. Thus, the selection of the dietary lipid supply 30 during early life is considered to be of great importance.

Because of the small size of their stomach and their limited tolerance of hypertonic foods, infants require a concentrated source of energy. Of the 3 nutrients supplying 35 energy, fat provides 9 kcal per gram, i.e. more than twice the energy present in carbohydrates or proteins. Most experts recommend that in infant and follow-on formulae fat should supply from 30% to 55% of the total energy. Preferably, the fats

used in the formulae of the invention are predominantly vegetable fats. However, whey and skim milk naturally contain traces of milk fat, and so a very small percentage of milk fat is likely to be present.

5 . Fatty acid composition of the diet determines fatty acid composition of all tissues, including storage tissues. The fat blend used in the formulae of the invention therefore preferably has an overall fatty acid composition as close as possible to that of human milk, in order to ensure similar membrane plasticity and same mobilization of energy in case of increased needs. Thus, the preferred fat blend supplies the essential fatty acids (linoleic and  $\alpha$ -linolenic acids), as well as adequate quantities of oleic acid, palmitic acid, lauric acid and myristic acid.

10 Human milk contains docosahexaenoic acid (DHA) and arachidonic acid (ARA) and thus breast-feeding provides infants with preformed LC-PUFAs. The DHA content of human milk varies considerably within populations and is strongly influenced by maternal diet. Globally, the DHA content of milk from mothers consuming Western diets ranges from 0.1 to 0.4%, with a mean of 0.25%, whereas in mothers consuming non-Western diets, the DHA content of milk is greater, ranging from 0.1 to 1.4%, with a mean of 0.6%. However, amounts of 0.2 to 0.3% are generally accepted as representative. The ARA content of human milk is less influenced by the diet than DHA. Globally, the ARA content of human milk from mothers consuming Western diets ranges from 0.2 to 0.7%, with a mean of 0.45%, whereas in mothers consuming non-Western diets, the ARA content ranges from 0.4 to 1.2%, with a mean of 0.6%. Both DHA and ARA levels are influenced by the duration of lactation and tend to decrease from colostrum to transitional and mature milk.

15 Accordingly, the lipid source of the present invention preferably also comprises at least one preformed LC-PUFA such as DHA. The source of the DHA may be a natural fish oil that also supplies eicosapentaenoic acid (EPA) with a DHA/EPA ratio > 4. Together with DHA, the lipid source may also include ARA, for example from fungal origin such as *Mortierella alpina*.

20 Formulae of the invention preferable have a reduced level of electrolytes compared to standard infant and follow-on formulas. For example, the Na/K ratio (mmol) may be around 0.4, the (Na+K)/Cl ratio (mmol) may be around 1.8, Na+K+Cl may be around 34 and (Na+K)-Cl may be around 10.

Formulae according to the present invention preferably have a low phosphate content. Preferably, the calcium content varies between 35 and 45 mg/100 mL, the phosphorus content varies between 15 and 25 mg/mL, and the Ca/P ratio is between 1.4 and 3.

5 The most preferred amounts are indicated in table 3 below.

Table 3

	invention (mg/100mL)	cow's milk (mg/100mL)	breast milk – average values (mg/100mL)
Calcium	41	120	30
Phosphorus	21	90	15
Ca / P ratio	2	1.3	2

10 The formulae according to the invention may also supply semi-essential nutrients which may be needed in particular conditions (e. g. taurine, nucleotides, carnitine, selenium).

15 Taurine is a free amino acid, which is not used to build up protein molecules. It has been shown to be involved in many physiological functions, e.g., as a trophic factor in the development of the central nervous system, maintaining the structural integrity of the membrane, regulating calcium homeostasis, as an osmolyte, a neuromodulator, and a neurotransmitter. It also conjugates with bile acids to form bile salts (essential for micelle formation and fat absorption).

20 Nucleotides are non protein nitrogen compounds which contain three characteristic components: a nitrogenous base, a sugar (ribose or deoxy-ribose), and one or more phosphate groups. Total nucleotide content in human milk represents 2 to 5% of the non-protein nitrogen. Cow's milk contains lower concentrations of nucleotides than human milk and its nucleotide profile differs markedly from that of human milk.  
 25 Addition of nucleotides in the present infant formula follows the physiological pattern of nucleotides levels in human milk, with a predominance of easily metabolised pyrimidines over less desirable purines: addition of nucleotides to the infant formula is safe. The levels of addition are within the range allowed by the European Union Scientific Committee for Food and the European Directive.

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Carnitine is a particular nitrogenous compound, which belongs to a group of food factors known as vitamin-like nutrients. It performs a crucial role in the energy supply of tissues during foetal life and in the neonatal period by facilitating the transport of long chain fatty acids into the mitochondria where beta-oxidation occurs. Fatty acids are indeed not able to pass in free form through the mitochondrial wall; the transfer into the mitochondria is governed by at least three enzymatic systems, namely carnitine – palmitoyl transferases I and II and carnitine -translocase, in which carnitine participates.

Thus, carnitine is required for proper lipid oxidation and carnitine deficiency or low carnitine intake can lead to impaired fat utilisation and altered lipid metabolism.

Carnitine has also a role in other metabolic processes, such as ketogenesis, lypolysis, and the maintenance of thermogenesis and nitrogen metabolism. Moreover, carnitine has been shown to improve utilisation of medium chain triglycerides in infants. Newborns have relatively low carnitine reserves and a very low activity of the enzyme catalysing the last step in the carnitine synthesis. Thus newborns are particularly at risk of becoming carnitine-deficient in the absence of an adequate supply of exogenous carnitine. Carnitine is preferably added to infant formulae, in order to reach a level close to that of human milk.

Formulae according to the invention may be in powder form or a ready to drink liquid. In the case of a powder formula, the following feeding table (table 4) may be used as a guide. However, the quantities may be changed according to medical advice. The introduction of an infant formula should be carried out under medical supervision. The standard reconstitution of formulae according to the invention is 12.9%, i.e. 12.9 g powder for 90 mL of water, which gives a caloric density of 67 kcal/100mL.

25

Table 4

Age of infant	quantity per feed		No. of feeds per day	
	Previously boiled water (mL)	number. of measuring scoops	Formula	Others
1 <sup>st</sup> and 2 <sup>nd</sup> weeks	90	3	6	-
3 <sup>rd</sup> and 4 <sup>th</sup> weeks	120	4	5	-
2 <sup>nd</sup> month	150	5	5	-
3 <sup>rd</sup> and 4 <sup>th</sup> months	180	6	5	-
5 <sup>th</sup> and 6 <sup>th</sup> months from the 7 <sup>th</sup> month onwards	210	7	5	-
	210	7	4-3	1-2

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In the case of a ready-to-drink liquid, special care needs to be taken to ensure that the probiotic does not accidentally come into contact with the liquid. Preferably, the probiotic is stored in powder form separate from the liquid, and is incorporated and homogenised into the liquid just before consumption, e.g. up to two hours before consumption.

The present invention also relates to a method of promoting physical development of an infant or a young child consisting in fully or partly feeding the infant or child with a formula according to the invention.

10

Formulae according to the invention have been shown to provide nutritional benefits including a better protein utilisation, a plasma amino acid pattern close to that of breast-fed infants, and adequate growth rates. The improved amino acid profile of formulae according to the invention results in better protein utilisation, as shown by the higher percentage of nitrogen retention found in infants fed with a formula according to the invention as compared with infants fed a regular whey-adapted formula (see table 5). As a result, the total amount of total nitrogen remains unchanged.

20

Table 5: nitrogen balance

	whey-adapted standard formulae	formulae according to the invention
absorption	89.5%	89.3%
retention	32.2%	39.6%

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Plasma amino acids in infants fed with a formula according to the invention have been shown to be closer to those of breast-fed infants compared to those in infants fed standard whey-adapted formulae. Furthermore, the protein content of formulae of the invention meets the needs of normal term infants during the first months of life without excessive energy intakes or increased body mass index. Still further, weight and length gains of infants fed with formulae according to the invention are comparable to breast-fed infants. (see Fig 1 to 3, and Example 1). In Fig. 1 and 2, white columns represent feeding with standard whey-adapted infant formulae, light grey columns feeding with human milk, and dark-grey columns represent feeding with a formula according to the invention. In Fig.3, black lozenges represent feeding with human milk, dark grey squares feeding with a formula according to the invention, light grey triangles feeding with standard whey adapted formulae.

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5 Formulae according to the invention present a reduced load on immature organs. Amino acids consumed in excess and not used for protein synthesis accumulate in the blood (hyperaminoacidemias) and are metabolised in the liver into urea which must be excreted through the kidneys, thus increasing kidney load. This unnecessary metabolic stress is well illustrated in infants fed standard whey-adapted formulae by plasma amino acid levels and plasma urea levels above those observed in breast-fed infants. The lower 10 protein content of formulae according to the invention obviates metabolic stress on infant immature organs due to excess dietary protein intake. This beneficial effect has been demonstrated: infants fed with a formula according to the invention have plasma urea nitrogen concentrations similar to those found in breast fed infants, and significantly lower than those found in the infants fed standard whey-adapted formulae. Plasma urea nitrogen is a very sensitive indicator of the adequacy of protein intake as higher levels than in breast-fed infants denote excess amino acids not utilised, whereas 15 lower levels denote insufficient protein supply. In addition, the modified sweet whey and probiotic(s) in formulae according to the invention may have a synergistic effect in promoting physical development particularly when LC-PUFA are also present.

20 According to a third aspect of the present invention, there is provided a method of improving gastro-intestinal comfort of an infant or a young child consisting in fully or partly feeding said infant or child with a formula according to the invention. In particular, an improvement in stool consistency, specifically a reduction of the frequency of hard stools, has been found in infants fed a formula according to the 25 invention. This improvement is thought to be due at least in part to a synergy between the probiotic(s) and the modified sweet whey proteins.

According to another aspect of the present invention, there is provided a method of developing a healthy gut microflora in an infant or a young child consisting in fully or partly feeding said infant or child with a formula according to the invention.

30 The gut microflora composition and the population size of strains is reported to be mainly regulated by competition for nutrients and oxygen availability. It is thought that factors in breast milk may be bifidogenic thus explaining the ease with which a favourable microflora is established in breast fed infants. Bifidobacteria, when growing, 35 use lactose as substrate to produce lactic and acetic acids that decrease the intestinal pH to 4-5. The low buffering capacity of breast milk would allow maintenance of such a

5 low pH, which inhibits the development of anaerobic putrefactive bacteria and enables the proliferation of Bifidobacteria and lactobacilli that are acid-tolerant. Without wishing to be bound by theory, the inventor believes that there is a synergy between the protein source in the formulae of the present invention and the probiotic such that a microflora similar to that found in breast fed babies is rapidly established and maintained in infants fed a formula according to the invention.

10 The reduced phosphate content of formula according to the first object of the present invention optimises bone formation and, together with lactose and low protein content, creates optimal condition for an intestinal flora with a predominance of Bifidobacteria.

### Examples

15 The following examples are illustrative of some of the products and methods of making the same falling within the scope of the present invention. They are not to be considered in any way limitative of the invention. Changes and modifications can be made with respect to the invention. That is, the skilled person will recognise many variations in these examples to cover a wide range of formulas, ingredients, processing, and mixtures to rationally adjust the naturally occurring levels of the compounds of the invention for 20 a variety of applications.

#### Example 1

25 The following example of a preferred formula according to the invention is illustrative only.

Nutrient	per 100kcal	per litre
Energy (kcal)	100	670
Protein (g) (Casein/Whey: 30/70)	1.83	12.3
Total Fat (g)	5.3	35.7
Of which		
Linoleic acid (g)	0.77	5.2
α-Linolenic acid (mg)	95	640
DHA(mg)	12	77
ARA	12	77
Lactose (g)	11.2	74.7
Minerals (g)	0.37	2.5
Na (mg)	23	150
K (mg)	89	590
Cl (mg)	64	430

Ca (mg)	62	410
P (mg)	31	210
Mg (mg)	7	50
Mn (µg)	8	50
Se (µg)	2	13
Vitamin A (µg RE)	105	700
Vitamin D (µg)	1.5	10
Vitamin E (mg TE)	0.8	5.4
Vitamin K1 (µg)	8	54
Vitamin C (mg)	10	67
Vitamin B1 (mg)	0.07	0.47
Vitamin B2 (mg)	0.15	1.0
Niacin (mg)	1	6.7
Vitamin B6 (mg)	0.075	0.50
Folic acid (µg)	9	60
Pantothenic acid (mg)	0.45	3
Vitamin B12 (µg)	0.3	2
Biotin (µg)	2.2	15
Choline (mg)	10	67
Fe (mg)	1.2	8
I (µg)	15	100
Cu (mg)	0.06	0.4
Zn (mg)	0.75	5

Bifidobacterium longum BB 536:

 $1 \times 10^7$  cfu/gram of dry product

Lactobacillus paracasei rhamnosus GG:

 $2 \times 10^7$  cfu/gram of dry product

## 5 Example 2

Over 100 infants whose mothers have elected not to breast feed after the 14<sup>th</sup> day of their life are enrolled in a randomised, controlled, double blind, multi-centre clinical trial of two groups. The first group is fed the formula of Example 1 and the second group is fed a similar formula but without probiotics. The following measurements, which are recognised parameters to assess the physical growth of infants, are made over 112 days (16 weeks):-mean weight gain in g/day, recumbent length, head circumference. Measurements are taken on recruitment then at 5 weeks, 8 weeks, 12 weeks and 16 weeks (in each case +/- 1 week) as follows:

15

Weight (to nearest 10 grams): infants are weighed without clothing on electronic weighing scales. The same scales are used for all infants at all visits. The electronic weighing scales are calibrated as per the manufacturer's recommendations at the start of the study and every 3 months thereafter until the end of the study.

20

Recumbent length (to nearest 1 mm): infants are measured using a standardised length board. At least two people are present to maintain proper body alignment and full body extension with feet flexed.

5 Head circumference (to nearest 1 mm): obtained using a standard non-elastic, plastic coated measuring tape. The measurement is taken approximately 2.5 cm above the eyebrows, directly over the largest circumference of the skull.

10 In addition, regular observations are made of digestive tolerance by observing stool characteristics, incidence of vomiting and regurgitation, frequency and duration of colic as follows:-

3 days after recruitment

3 days before and after anthropometric measurements at 5 weeks, 8 weeks and 12 weeks

3 days before final anthropometric measurements at 16 weeks

15 In addition, frequency of episodes of morbidity are noted (number of times seen by healthcare professionals plus episodes of ill health, particularly gastrointestinal conditions) are noted. These observations are made by the infants' care givers in a diary provided for the purpose.

20 It is found that infants fed the formula of the invention display satisfactory physical development coupled with improved gastrointestinal comfort (as demonstrated by the observations of digestive tolerance detailed above) when compared with the control group.

25

**Claims**

1. Infant or follow-on formula comprising a source of proteins, a source of lipids, a source of carbohydrates and a probiotic, wherein at least 40% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.  
5
2. Formula according to claim 1 wherein at least 60% of the proteins are modified sweet whey proteins comprising no CGMP or reduced CGMP.
- 10 3. Formula according to claim 1 or 2, wherein the probiotic is a *Bifidobacteria* or a *Lactobacillus*.
4. Formula according to claim 3 wherein the *Bifidobacteria* is *Bifidobacterium longum BB 536*.  
15
5. Formula according to claim 3 wherein the *Lactobacillus* is *Lactobacillus paracasei rhamnosus GG*.
6. Formula according to claim 1 or 2 which contains both a *Bifidobacterium* and a *Lactobacillus*.  
20
7. Formula according to claim 6 wherein the *Bifidobacteria* is *Bifidobacterium longum BB 536* and the *Lactobacillus* is *Lactobacillus paracasei rhamnosus GG*.
- 25 8. Formula according to any preceding claim wherein the proteins are intact.
9. Formula according to any of claims 1 to 7 wherein the proteins are partially hydrolysed.
- 30 10. Formula according to any preceding claim wherein the proteins are present in a maximum proportion of 2g/100 kcal, preferably 1.85, most preferably between 1.8 and 1.85 g/100 kcal.
- 35 11. Formula according to preceding claim wherein the whey proteins with reduced CGMP represent at least 60% of the total proteins, preferably at least 70% of the total proteins.

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12. Formula according to any preceding claim further comprising at least one LC-PUFA.
- 5      13. Formula according to claim 12 wherein the LC-PUFA comprises DHA, associated or not with ARA.
14. Formula according to one of claims 1 to 13 further having a low amount of electrolytes.
- 10     15. Method of promoting physical development of an infant or a young child consisting in fully or partly feeding said infant or child with a formula comprising a source of proteins, a source of lipids, a source of carbohydrates and at least one probiotic, wherein at least 40% of the proteins are whey proteins comprising no CGMP or reduced CGMP.
- 15     16. Method according to claim 15 using a formula according to any of claims 1 to 14.
17. Method according to claim 15 or claim 16 wherein the physical development of the infant or child is characterised by a reduced load on immature organs.
- 20     18. Method of improving gastro-intestinal comfort of an infant or a young child consisting in fully or partly feeding said infant or child with a formula comprising a source of proteins, a source of lipids, a source of carbohydrates and at least one probiotic, wherein at least 40% of the proteins are whey proteins comprising no CGMP or reduced CGMP.
- 25     19. Method according to claim 18 using a formula according to any of claims 1 to 14.
- 30     20. Method of developing a healthy gut microflora in an infant or a young child consisting in fully or partly feeding said infant or child with a formula comprising a source of proteins, a source of lipids, a source of carbohydrates and at least one probiotic, wherein at least 40% of the proteins are whey proteins comprising no CGMP or reduced CGMP.
- 35     21. Method according to claim 20 using a formula according to any of claims 1 to 14.

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP2004/006614

A. CLASSIFICATION OF SUBJECT MATTER  
IPC 7 A23L1/29 A23L1/30 A23L1/305 A61K35/74

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, FSTA, WPI Data, PAJ

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Y	US 2003/077255 A1 (SABHARWAL HEMANT K) 24 April 2003 (2003-04-24) page 2, paragraph 14; claims 1-12	1-21
		-/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

## \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the International filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the International filing date but later than the priority date claimed

\*T\* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the International search

Date of mailing of the International search report

30 September 2004

11/10/2004

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## INTERNATIONAL SEARCH REPORT

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International Application No  
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**C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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3

**INTERNATIONAL SEARCH REPORT**

International application No. <b>PCT/EP2004/006614</b>
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**Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: **15–21 (partly)**  
because they relate to subject matter not required to be searched by this Authority, namely:  
**Although claims 15–21 are directed to a method of treatment of the human body, the search has been carried out and based on the alleged effects of the compound/composition.**
2.  Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.  Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this International application, as follows:

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No  
PCT/EP2004/006614

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